

**Mass Spectral identification and quantitation of Single Ion Chromatography of unresolved limonene, sylvestrene and  $\beta$ -phellandrene in the leaf volatile terpenoids of *Pinus peuce* (Bulgaria) and comparison with oils from other regions**

**Robert P. Adams**

Biology Department, Baylor University, Waco, TX 76798 robert\_adams@baylor.edu

and

**Alexander N. Tashev**

University of Forestry, Dept. of Dendrology  
10, Kliment Ochridsky Blvd., 1797 Sofia, Bulgaria

**ABSTRACT**

Analyses of the leaf volatile oils of *Pinus peuce* from Bulgaria revealed the oil is dominated by  $\alpha$ -pinene (27.1-49.2%), camphene (2.8-8.6%),  $\beta$ -pinene (9.7-20.4%), and germacrene D (8.7-10.6%). Sylvestrene varied considerable from 1.5 to 34.9%. Two plants were very high (12.5, 34.9%) and three plants were low in sylvestrene (1.5, 1.9, 2.0%), suggesting chemotypes are present. Comparisons with *P. peuce* from Montenegro-Serbia, Serbia, Macedonia and Greece showed the compositional averages appeared to be fairly uniform in the Balkans, except for the literature reports of limonene, sylvestrene and  $\beta$ -phellandrene. Bulgaria  $\alpha$ -pinene (38.8%) is similar to oils from other regions (Table 1), although Karapandzova et al. (2011) reported a population with only 9.2%  $\alpha$ -pinene. Camphene (6.1%) was similar to other regions.  $\beta$ -pinene was variable among regions with Bulgarian oil being high (16.2%), but not as high as reported from Greece (22.0) by Koukos et al. (2000). The amounts of limonene, sylvestrene and  $\beta$ -phellandrene were inconsistent (Table 1) and this probably reflects both the difficulty of identification and the lumping of compounds into one value in some reports. Published on-line [www.phytologia.org](http://www.phytologia.org) *Phytologia* 101(2): 120-130 (June 21, 2019). ISSN 030319430.

**KEY WORDS:** *Pinus peuce*, Bulgaria, volatile leaf oil, terpenes, composition, mass spectra limonene, sylvestrene,  $\beta$ -phellandrene, Single Ion Chromatograms.

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The advances in capillary column technology have made the analysis and identification of volatile oil components from plants relatively easy (Adams 2007). The most commonly used capillary column is bonded, 5% phenyl 95% dimethylpolysiloxane due to its excellent resistance to degradation and range of use from ambient to 325° C (350° C max). When I (Adams) began to assemble a terpene library in 1976 in Dr. Ernst von Rudloff's laboratory, we used only carbowax columns (PEG, polyethylene glycol) which were short lived due to degradation by oxygen and water. The advent of bonded, 5% phenyl 95% dimethylpolysiloxane capillary columns was championed by Walter Jennings who co-founded J & W Scientific in 1974. Soon J & W released the DB-5 column (Duro Bond 5% phenyl 95% dimethylpolysiloxane). DB-5 is equivalent to: Rtx-5 (Restek), G27, G36 (USP nomenclature), HP-5, CP-Sil 8 CB (Agilent), BP-5 (SGE), ZB-5 (Phenomenex), Optima-5 (Machery-Nagel), SPB-5 (Supleco), EC-5, AT-5 (Alltech) and 007-5 (Quadrex). I (Adams) immediately seized on the opportunity and adopted DB-5 as the standard column for volatile oil analyses for use in building the Adams Terpene Library (Adams 2007, 4<sup>th</sup> ed.), in spite of the almost universal utilization of carbowax (PEG) columns in the fragrance and flavors industry at the time. No column is capable of resolving all components in all volatile oils. Systems are now available for simultaneous co-chromatography on a non-polar (ex. DB-5) and a polar (ex. PEG) column. But, a vast number of labs still use DB-5 (or equivalent) as their primary column. However, there is a very common, and thus, important duo or trio of compounds that co-elute: limonene, sylvestrene and  $\beta$ -phellandrene. They are difficult to identify and

quantify on DB-5. These three compounds are found in the leaf volatile oils of several *Pinus* species (Ioannou et al. 2014) and are sometimes just reported together (limonene- $\beta$ -phellandrene, Karapandzova, et al. 2011).

Single Ion(s) Chromatograms (SIC) is a powerful tool available on mass spectrometers. SIC can be used to identify and quantitate co-eluting compounds such as limonene-sylvestrene- $\beta$ -phellandrene;  $\alpha$ -cedrene- $\beta$ -funebrene; and cedrol-widdrol. In a study of *Pinus peuce* in Bulgaria, we found a tree that had limonene (trace), sylvestrene (12.5%) and  $\beta$ -phellandrene (0.7%) in its oil and presented an opportunity to carefully investigate the feasibility of utilizing SIC for identification and quantitation.

*Pinus peuce* Griseb. is endemic to the Balkan peninsula (Serbia, Montenegro, Albania, Macedonia and Bulgaria) Gymnosperm Database (2019). There are several reports of the leaf (needle) essential oil composition of *Pinus peuce* (Ioannou et al. 2014; Karapandzova et al. 2010, 2011; Koukos et al. 2000; Mitic et al. 2017; Nicolic et al. 2014; Petrakis et al. 2001. Mitic et al. (2017) has given an excellent, recent review *P. peuce* taxonomy, history and essential oil analyses, so the reader is referred to that paper.

The purposes of this paper are to report on an investigation of the feasibility of utilizing SIC for identification and quantitation of limonene-sylvestrene- $\beta$ -phellandrene, and present a complete analysis of the volatile leaf of *Pinus peuce* from Bulgaria and compare its composition with reports from the Balkans.

## MATERIALS AND METHODS

Leaf samples of *P. peuce* collected: Bulgaria, Pirin Mountain (North), National Park "Pirin". Between the huts "Banderitza" and "Vihren", in the valley of Banderishka river, growing with *Pinus heldreichii*, *P. mugo*, *Juniperus communis*, 41°45'57.7" N, 23°25'25.1" E., 1831 m, Date 18.05.2015. Coll. Alexander & Nikolay Tashev 2015 Sp. 1-5 PP1-P5, Lab Acc. Robert P. Adams 14737-14741. Voucher specimens are deposited at University of Forestry, Dept. of Dendrology, Sofia, Bulgaria.

Gently dried leaves (100g, 40 - 45°C) were cut into 3 cm lengths to promote the release of terpene oils and then immediately steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Limonene, sylvestrene and  $\beta$ -phellandrene eluted as a single peak on DB-5, but their amounts were quantitated by use of Single Ion Chromatograms using ions: 67, 68, 77, 79, and 93. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

## RESULTS AND DISCUSSION

Mass spectra of pure limonene, sylvestrene and  $\beta$ -phellandrene are shown in Fig. 1(modified from Adams, 2007, p. 143). Colored ellipses indicate the critical mass pairs that resolve these three compounds. The following is a schematic for identification of limonene (LMNN), sylvestrene (SYLV) and  $\beta$ -phellandrene (BPHL):

**A. Retention times** (time, KI): LMNN (8.69, 1029), SYLV (8.80, 1027), BPHL (8.82, 1029). If only LMNN and BPHL are present, and neither are large components, they can sometimes be resolved, but if not resolved, **then use single ions as:**

**B. Mass Ion 93:** Note that pure LMNN (Fig. 1) has 68>93, but if LMNN is just a trace (as in *P. peuce*, 14740), even the 'purest' spectrum of LMNN may not have 68>93 (see Fig. 2a, where 68 is only ½ the size of 93, due to the presence of considerable sylvestrene).

However, 93 is very useful when there is partial peak separation between LMNN, SYLV, and/ or BPHL.

**C. Next, consider Mass Ions 77, 79:** If 77>79, this implies BPHL is present (Fig. 1); So, check the mass spectrum in your reference library, it should be  $\beta$ -phellandrene (cf. Fig. 1).

If 79>77, this implies LMNN or SYLV is present as both have ion 79>77. (of course, there may be another unidentified compound present, never forget this.)

**D. Check if ion 68>93** for LMNN (but this did not work for plant 14740, because of the large amount of sylvestrene that contributes ion 93 (Fig. 1).

**E. Then, check if 68>67 or if 67 ~ 68.** Checking 67 vs. 68 in individual scans from about 8.680 mins thru 8.698 revealed that ion 67 gradually increased and the ratio of 67/68 increased such that the height of 67 was approximately equal to 68 (i.e, 67~68) at 8.698, as one would expect to find in sylvestrene (compare 67/68 in Fig. 2a and 2b).

**F. In addition, one can check other trends of ratios:** we found that the ratio of 68/93 declined from 8.680 mins thru 8.698 due to increases in the amount of sylvestrene (cf. compare 68/93 in Fig. 2a and 2b). Clearly trace amounts are very difficult to detect, but can be detected by very careful single ion analyses.

The SIC region from 8.754 to 8.809 was nearly pure sylvestrene and constitutes a large majority of the SIC peak. In fact, there is mostly sylvestrene from 8.698 thru 8.809 and it is estimated to be 95% of the peak (Fig. 2b, upper).

The amount of  $\beta$ -phellandrene (from 8.828 to 8.846 mins) is estimated at about 5% of the SIC peak (Fig. 2c, upper). Although these estimates of limonene, sylvestrene and  $\beta$ -phellandrene are just estimates, it seems prudent to utilize these mass spectral methods to report composition, rather than lump the components into one sum.

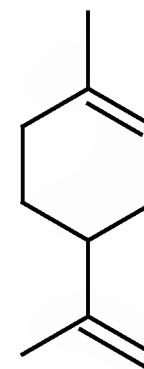
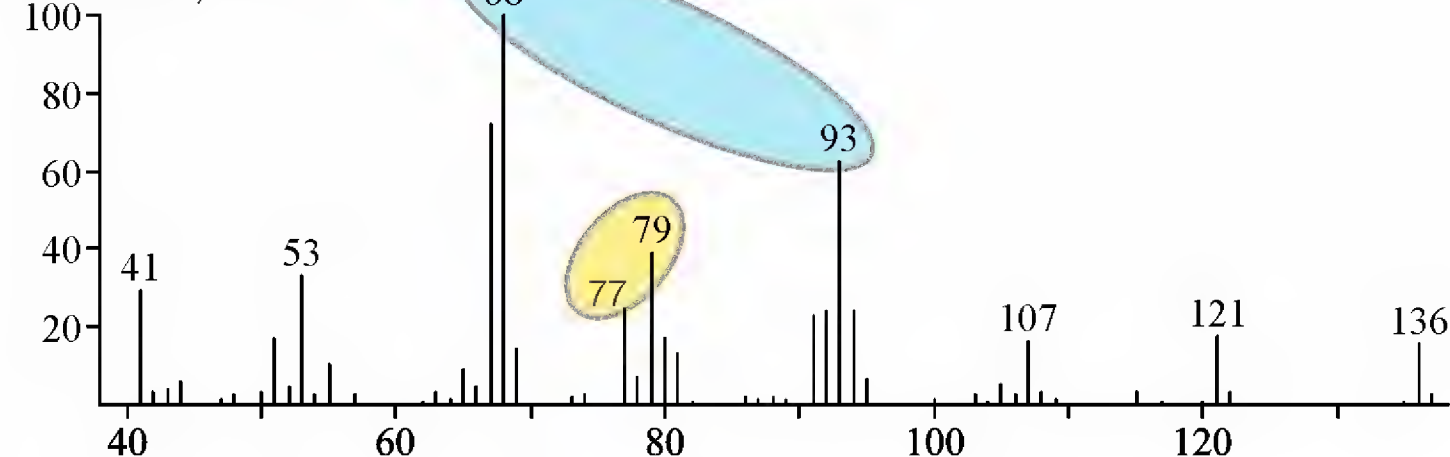
## COMPARISON OF THE OIL OF *P. PEUCE* FROM BULGARIA WITH OTHER REGIONS

The composition of the volatile leaf essential oils of *P. peuce* from Bulgaria are shown in Table 1 and compared with Montenegro-Serbia (Mitic et al. 2017), Serbia (Nikolic et al. 2014), Macedonia (Karapandzova et al. 2011) and Greece oils (Koukos et al. 2000; Petrakis et al. 2001). Bulgaria  $\alpha$ -pinene (38.8%) is similar to oils from other regions (Table 1), although Karapandzova et al. (2011) reported a population with only 9.2%  $\alpha$ -pinene. Camphene (6.1%) was similar to other regions.  $\beta$ -pinene was variable among regions with Bulgarian oil being high (16.2%), but not as high as reported from Greece (22.0%) by Koukos et al. (2000).

RT: 8.69 AI: 1024 KI: 1029 **Limonene**  
 CAS#: 138-86-3 MF: C<sub>10</sub>H<sub>16</sub> FW: 136 MSD LIB#: 164  
 CN: methyl-4-(1-methylethenyl)cyclohexene <1->  
 Synonyms: cajeputene; cinene; kautschin; nesol; p-mentha-1,8-diene; dipentene  
 Source: distillation of sweet anis leaves. Calif., RP Adams #5452; 96.49% *Citrus sinensis*,  
 Zhiwu, Xuebao, 30:623(1988); 96.41% *Citrus aurantium*; 95.00% *Citrus deliciosa*

**8.69 LIMONENE**

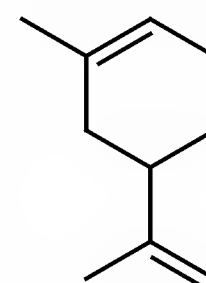
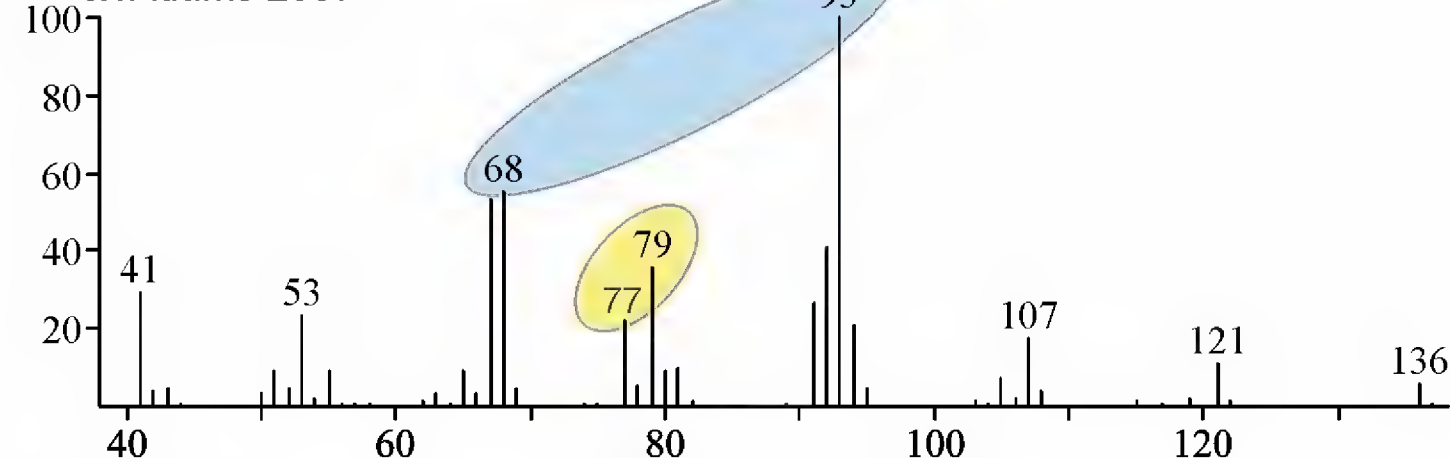
ex Adams, 2007



RT: 8.80 AI: 1027 KI: 1030 **Sylvestrene**  
 CAS#: 1461-27-4 MF: C<sub>10</sub>H<sub>16</sub> FW: 136 MSD LIB#: 1453  
 CN: cyclohexene, 1-methyl-5-(1-methylethenyl)-  
 Synonyms: m-mentha-1(6),8-diene; Source: ref. cpd. ex E. von Rudloff

**8.73 SYLVESTRENE**

ex Adams 2007



RT: 8.82 AI: 1028 KI: 1029 **Phellandrene <math>\beta</math>->**  
 CAS#: 555-10-2 MF: C<sub>10</sub>H<sub>16</sub> FW: 136 MSD LIB#: 161  
 CN: methyl-6-(1-methylethyl)cyclohexene <3->; Synonyms: p-menth-1(7),2-diene  
 Source: distillation of sweet anis leaves(Calif.), RP Adams #5452; 63.05% *Levisticum officinale*,  
 p. 641, in: Flav. & Frag.: A World Persp., Lawrence, et al., Elsevier Science Publishers,  
 Amsterdam(1988); 61.00% *Angelica archangelica*; 21.00% *Lavandula pinnata*

**8.70 PHELLANDRENE<BETA->**

ex Adams 2007

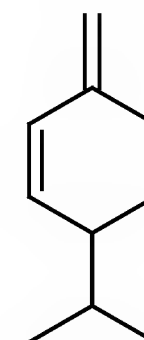
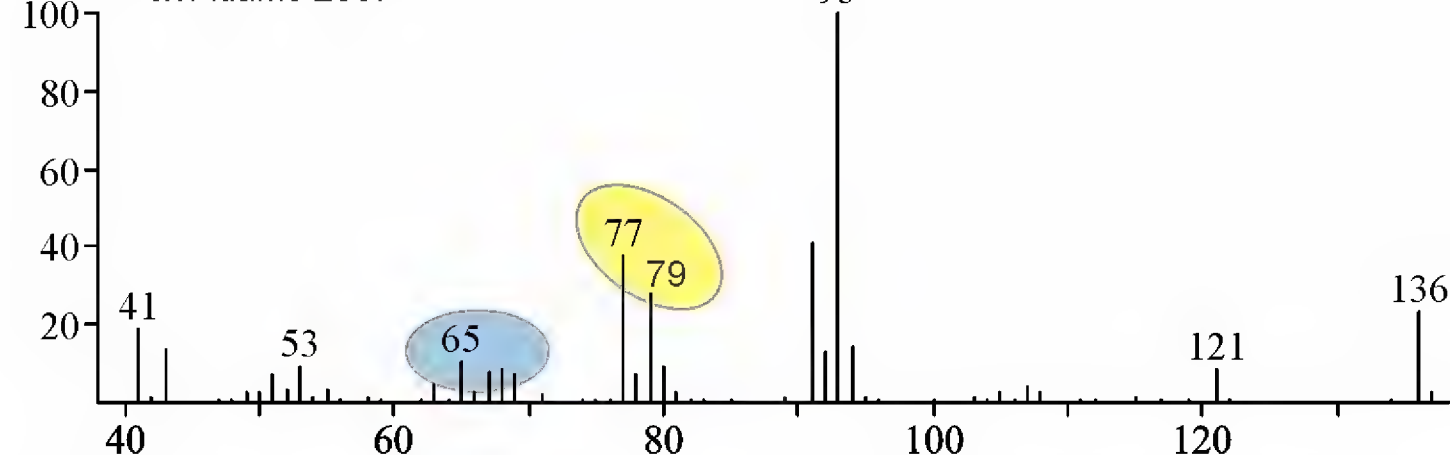


Figure 1. Mass spectra of limonene, sylvestrene and  $\beta$ -phellandrene (ex Adams 2006). Circles indicate diagnostic mass pairs for identification and quantification.



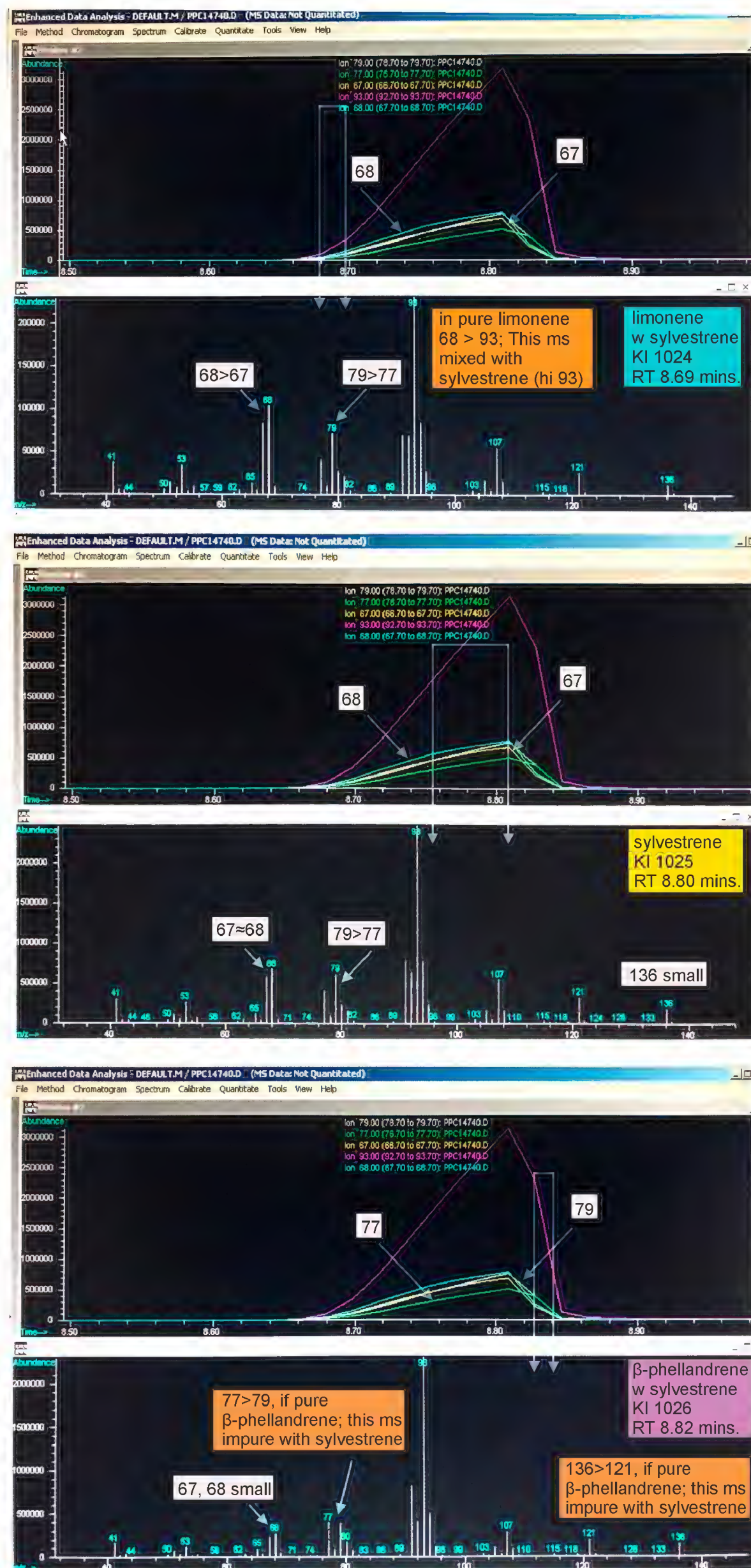


Fig. 2. Single Ions Chromatograms for ions 93, 79, 77, 68, and 67 (superimposed, for 8.5 - 9.0 mins.)

Fig. 2a. MS of limonene (lower section) from the average of 8.680 to 8.698 mins in SIC region as shown by the 2 arrows (upper). The MS has some sylvestrene contamination. Note the point of inflection in ion 93 at about 8.698, indicating an increase in sylvestrene. Limonene is only a trace of the SIC peak. (cf. reference MS in Fig. 1)

Fig. 2b. The MS of sylvestrene (lower section) from the average of 8.754 to 8.809 mins in SIC region as shown by the 2 arrows (upper). Notice (in the SIC), the maximum amounts of ions 67, 68, 79 at 8.809, revealing the peak top of sylvestrene. This is a very pure sylvestrene MS (cf. reference MS in Fig. 1). Sylvestrene is about 95% of the SIC peak.

Fig. 2c. The MS of  $\beta$ -phellandrene (lower section) from the average of 8.828 to 8.846 mins in SIC region as shown by the 2 arrows (upper). The MS contains some sylvestrene contamination (cf. reference pure  $\beta$ -phellandrene MS in Fig. 1). Note the sharp decline in ion 67, signaling the end of the sylvestrene peak.  $\beta$ -phellandrene is about 5% of the SIC peak.



The amounts of limonene, sylvestrene and  $\beta$ -phellandrene were inconsistent (Table 1) and this probably reflects both the difficulty of identification and the lumping of compounds into one value in some reports. The Bulgaria oil consist of trace, 9.5 and 1.0% of limonene, sylvestrene and  $\beta$ -phellandrene (Table 1). Aside from the present report, sylvestrene has only reported by Ioannou et al. (2014) (Table 1). But, Karapandzova et al. (2011) list limonene +  $\beta$ -phellandrene as 2.0-6.6%, with no sylvestrene reported, so it seems likely their report is limonene + sylvestrene +  $\beta$ -phellandrene. Petrakis (Greece) reported only limonene (2.5%), with no sylvestrene or  $\beta$ -phellandrene, but because our Bulgaria plants are not far away from the Greece plant examined by Petrakis, and only one plant contained a trace amount of limonene, it seems likely that Petrakis (Greece) oil is mostly sylvestrene (~2.5%). Most studies found (1.0-6.8%)  $\beta$ -phellandrene, however, Petrakis and Ioannou reported no  $\beta$ -phellandrene in oils from Greece, nor from *P. peuce*, cultivated in Finland (Table 1). Clearly there is some confusion on the amounts of these compounds in some reports. Hopefully, the mass spectral methods presented in this paper will be of use to correct these reports in the future.

Generally, the Bulgarian oil composition is similar to that from other regions (Table 1). However, there are a few unusual amounts that are noted with ?? in table 1. Mitic et al. (2017) reported 1.1%  $\beta$ -gurjunene and 0.6% aromadendrene. Karapandzova et al. (2011) listed  $\beta$ -selinene as 0 to 0.4%. Petrakis et al. (2001) reported neryl acetate (0.9%), phenyl ethyl isovalerate (4.1%), and aristolene (0.6%), and these were not found in any other oils (Table 1). Koukos et al. (2000) reported 13.4% citronellol, and 0.0% germacrene D which are at odds with other reports.

#### VARIATION IN THE OIL COMPOSITION AMONG TREES IN BULGARIA

The major components were  $\alpha$ -pinene (27.1-49.2%), camphene (2.8-8.6%),  $\beta$ -pinene (9.7-20.4%), myrcene (1.4-4.1%), sylvestrene (1.5-34.9%),  $\beta$ -phellandrene (0-1.4%), bornyl acetate (1.8-7.0%), (E)-caryophyllene (1.8-2.4%), germacrene D (8.7-10.6%) and germacrene-D-4-ol (0.3-1.1%).

The variation in sylvestrene was greatest with plants 14738 and 14740 having 34.9 and 12.5% in contrast to the other three plants that range from only 1.5-2.0%. This may constitute a sylvestrene chemotype (high and low) in *P. peuce*, but additional sampling is needed to confirm this.

#### ACKNOWLEDGEMENTS

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Table 1. The leaf oil of *Pinus peuce* from Bulgaria compared with published analyses. Major compounds in bold face yellow. Compositional values less than 0.1% are denoted as traces (t). ?? indicates a possible mis-identification in the literature. Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column.

KI	compound	Bulgaria this study 15775	M-Serbia Mitic <sup>1</sup>	Serbia Nikolic <sup>2</sup>	Macedonia Karapand- zova <sup>3</sup>	Greece Petrakis <sup>4</sup>	Greece, Koukos <sup>5</sup>	cult Finland Ioannou <sup>6</sup>
921	tricyclene	0.4	-	0.7	t	-	0.4	0.5
924	$\alpha$ -thujene	-	0.6	t	t	0.4	-	-
<b>932</b>	<b><math>\alpha</math>-pinene</b>	<b>38.8</b>	<b>36.5</b>	<b>45.5</b>	<b>9.2-27.3</b>	<b>21.8</b>	<b>23.1</b>	<b>30.1</b>
946	camphene	6.1	8.5	10.0	0.9-6.4	2.5	5.5	5.9
948	benzaldehyde	-	-	-	t-1.4	-	-	-
967	(tert-butyl benzene)	-	-	-	0-7.8	-	-	-
<b>969</b>	<b>sabinene</b>	<b>t</b>	<b>0.7</b>	<b>t</b>	<b>0-0.3</b>	<b>0.3</b>	-	-
<b>974</b>	<b><math>\beta</math>-pinene</b>	<b>16.2</b>	<b>6.8</b>	<b>10.8</b>	<b>6.0-13.1</b>	<b>9.4</b>	<b>22.0</b>	<b>10.8</b>
988	myrcene	2.7	1.0	0.9	t-1.4	4.0	2.0	1.5
1002	$\alpha$ -phellandrene	0.3	0.8	0.2	t-0.8	0.2	0.2	0.5
<b>1008</b>	<b><math>\delta</math>-3-carene</b>	<b>0.2</b>	-	t	0-1.7	0.1	0.5	0.1
1014	$\alpha$ -terpinene	t	-	t	0-1.2	0.2	0.3	0.1
1020	p-cymene	t	-	t	t-0.8	-	0.3	-
1024	limonene	t	-	-	?	2.5 ??	-	-
<b>1025</b>	<b>sylvestrene</b>	<b>9.5</b>	<b>??</b>	<b>??</b>	<b>??</b>	<b>??</b>	<b>??</b>	<b>4.7</b>
<b>1025</b>	<b><math>\beta</math>-phellandrene</b>	<b>1.0</b>	<b>4.7</b>	<b>3.4</b>	<b>2.0-6.6</b> <b>Lmn+<math>\beta</math>ph</b>	<b>- ??</b>	<b>6.8</b>	-
<b>1044</b>	<b>(E)-<math>\beta</math>-ocimene</b>	<b>t</b>	-	t	0-0.3	0.7	-	t
1054	$\gamma$ -terpinene	t	-	t	0-0.4	0.2	-	t
1065	cis-sabinene hydrate	t	-	-	-	-	-	0.3
1082	m-cymenene	-	-	-	-	-	-	-
<b>1086</b>	<b>terpinolene</b>	<b>0.5</b>	<b>0.5</b>	<b>0.3</b>	<b>0.3-0.7</b>	<b>0.6</b>	<b>- ??</b>	<b>0.9</b>
1095	linalool	-	-	-	-	t	-	-
1114	endo-fenchol	t	-	-	-	t	-	-
1118	cis-p-menth-2-en-1-ol	0.2	-	-	-	-	-	-
1122	$\alpha$ -campholenal	t	-	-	t-0.1	-	-	-
1135	trans-pinocarveol	-	-	t	0-0.3	-	-	-
1136	trans-p-menth-2-en-ol	0.2	-	-	-	-	-	-
1136	trans-sabinol	0	-	-	-	-	-	-
1141	camphor	0.3	-	-	0-0.2	-	-	-
1165	borneol	0.6	-	t	0-2.8	t	-	-
1174	terpinen-4-ol	t	-	t	0-0.1	0.1	-	0.1
1179	p-cymen-8-ol	t	-	-	0-0.1	-	-	-
1186	$\alpha$ -terpineol	0.2	-	-	0.1-0.4	0.5	-	-
1195	myrtenol	t	-	t	-	-	0.2	-
1195	cis-piperitol	t	-	-	-	-	-	-
1207	trans-piperitol	0.1	-	-	-	-	-	-
1215	trans-carveol	t	-	-	0-0.1	-	-	-
1218	endo-fenchyl acetate	t	-	t	0-0.1	0.3	-	-
1223	citronellol	-	-	-	-	-	<b>13.4 ??</b>	-
1226	cis-carveol	t	-	-	-	-	-	-
1243	piperitone	t	-	-	t	-	-	-
1253	trans-sabinene hydrate acetate	-	-	0.1	-	-	-	-
1254	linalool acetate	t	-	-	t	-	0.2	-
<b>1284</b>	<b>bornyl acetate</b>	<b>4.5</b>	<b>6.8</b>	<b>4.5</b>	<b>0.2-11.7</b>	<b>- ??</b>	<b>9.8</b>	<b>6.4</b>
1342	trans-piperitol acetate	0.4	(1.6?)	0.8	-	-	-	-
<b>1345</b>	<b><math>\alpha</math>-terpinyl acetate</b>	<b>0.8</b>	<b>0.9</b>	<b>0.9</b>	<b>0-3.0</b>	<b>0.2</b>	<b>2.0</b>	<b>0.2</b>
1348	$\alpha$ -cubebene	-	-	-	-	-	-	0.4
1359	neryl acetate	-	-	-	-	<b>0.9 ??</b>	-	-
1374	$\alpha$ -copaene	t	-	t	t-0.2	-	-	0.4
1379	geranyl acetate	-	-	-	t	0.4	-	-
1387	$\beta$ -bourbonene	t	-	0.1	0.1-0.3	-	-	0.2
1389	$\beta$ -elemene	t	-	0.2	0.1-1.3	-	-	0.6
1403	methyl eugenol	-	-	-	t	-	-	-
1407	longifolene	t	-	-	t	-	-	-
1409	$\alpha$ -gurjunene	-	-	-	-	1.0	-	-
<b>1417</b>	<b>(E)-caryophyllene</b>	<b>2.3</b>	<b>5.2</b>	<b>3.0</b>	<b>3.9-7.1</b>	<b>4.7</b>	<b>3.0</b>	<b>9.8</b>



1430	$\beta$ -copaene	t	-	0.2	0.2-0.9	-	-	0.1
KI	compound	Bulgaria this study 15775	M-Serbia Mitic <sup>1</sup>	Serbia Nikolic <sup>2</sup>	Macedonia Karapand- zova <sup>3</sup>	Greece Petraakis <sup>4</sup>	Greece, Koukos <sup>5</sup>	cult Finland Ioannou <sup>6</sup>
1431	$\beta$ -gurjunene	-	<b>1.1 ??</b>	-	-	-	-	-
1439	aromadendrene	t	<b>0.6 ??</b>	-	t	-	-	-
1454	$\alpha$ -humulene	0.4	0.8	0.5	0.5-1.6	0.9	0.5	1.8
1454	(E)- $\beta$ -farnesene	-	-	t	-	-	-	-
1478	$\gamma$ -muurolene	-	0.6	-	0.1-1.2	-	-	t
<b>1480</b>	<b>germacrene D</b>	<b>9.7</b>	<b>11.4</b>	<b>11.1</b>	<b>7.1-19.9</b>	<b>18.8</b>	<b>- ??</b>	<b>17.0</b>
1489	$\beta$ -selinene	t	-	-	0-0.4	-	-	-
1495	phenyl ethyl isovalerate				-	<b>4.1 ??</b>	-	
1500	bicyclogermacrene	0.6	-	0.6	0.6-2.0	-	-	1.2
1500	$\alpha$ -muurolene	0.1	1.2	t	0-1.9	1.0	0.3	0.2
1508	germacrene A	0.1	-	-	-	-	-	0.1
1513	$\gamma$ -cadinene	0.2	0.5	-	0.4-2.7	-	0.4	t
1522	$\delta$ -cadinene	0.4	0.8	0.1	1.5-8.3	3.7	0.7	0.5
1537	$\alpha$ -cadinene	t	-	t	0.1-0.3	-	-	-
1561	(E)-nerolidol	-	-	-	t-3.1	-	-	-
<b>1574</b>	<b>germacrene-D-4-ol</b>	<b>0.6</b>	<b>0.5</b>	<b>0.9</b>	<b>0-3.7</b>	<b>-</b>	<b>-</b>	<b>t</b>
1583	caryophyllene oxide	0.1	-	t	0-0.7	-	-	-
1608	humulene epoxide II	t	-	-	0-0.4	-	-	-
1608	$\beta$ -atlantol	t	-	-	-	-	-	-
1638	epi- $\alpha$ -cadinol	t	-	-	-	-	-	0.5
1640	epi- $\alpha$ -muurolol	t	-	-	-	-	-	-
1644	$\alpha$ -muurolol	-	-	-	-	-	-	0.5
1645	1-epi-cubenol	t	-	-	t-0.3	-	-	t
1652	$\alpha$ -cadinol	0.4	-	0.1	0.3-7.0	-	-	-
1685	germacra-4(15),5,10)14)- trien-1-al	t	-	0.2	-	-	-	-
1739	oplopanone	0.1	-	t	t	-	-	-
1759	benzyl benzoate	-	-	-	t	-	-	-
1762	aristolene	-	-	-	-	<b>0.6 ??</b>	-	-
1959	hexadecanoic acid	0.2	-	-	-	-	-	-
1987	manool oxide	t	-	-	-	-	-	t
2014	palustradiene	t	-	-	-	-	-	-
2056	abietatriene	-	-	-	t	-	-	-
2087	abietadiene	-	-	-	t	-	-	-
2149	abienol	0.2	-	t	t	-	-	-
2220	isopimaral	t	-	-	-	-	-	-
<b>2243</b>	<b>palustral (8,13- abietadien-18-al</b>	<b>0.2</b>	-	-	-	-	-	-
2274	dehydro abietal	-	-	-	t	-	-	0.1
2310	isopimarol	-	0.0	-	-	-	-	-
2312	abieta-7,13-dien-3-one	-	-	-	-	-	-	-
2313	abietal	t	-	t	0-3.7	-	-	-

<sup>1</sup>Mitic et al. 2017; <sup>2</sup>Nicolic et al. 2014; <sup>3</sup>Karapandozova, et al. 2011; <sup>4</sup>Petraakis et al. 2001; <sup>6</sup>Ioannou et al. 2014; <sup>5</sup>Koukos et al. 2000.

Table 2. Variation in constituents of the leaf volatile oil of *P. peuce* within a population in Bulgaria. Major and diagnostic compounds are in bold face and yellow.

KI	compound	Bulgaria 14738	Bulgaria 14740	Bulgaria 14741	Bulgaria 14737	Bulgaria 14739	Average 15775
<b>921</b>	<b>tricyclene</b>	<b>0.2</b>	<b>0.5</b>	<b>0.5</b>	<b>0.5</b>	<b>0.2</b>	<b>0.4</b>
<b>924</b>	<b><math>\alpha</math>-thujene</b>	-	-	-	-	-	-
<b>932</b>	<b><math>\alpha</math>-pinene</b>	<b>27.1</b>	<b>31.4</b>	<b>36.4</b>	<b>47.4</b>	<b>49.2</b>	<b>38.8</b>
<b>946</b>	<b>camphene</b>	<b>2.8</b>	<b>7.9</b>	<b>8.6</b>	<b>8.1</b>	<b>2.9</b>	<b>6.1</b>
<b>969</b>	<b>sabinene</b>	t	t	t	t	t	t
<b>974</b>	<b><math>\beta</math>-pinene</b>	<b>11.0</b>	<b>18.3</b>	<b>19.1</b>	<b>9.7</b>	<b>20.4</b>	<b>16.2</b>
988	myrcene	2.3	1.4	2.7	4.1	2.9	2.7
1002	$\alpha$ -phellandrene	0.4	0.4	0.3	0.3	0.2	0.3
<b>1008</b>	<b><math>\delta</math>-3-carene</b>	<b>0.9</b>	<b>0.1</b>	t	<b>0.3</b>	t	<b>0.2</b>
1014	$\alpha$ -terpinene	t	t	t	t	t	t
1020	p-cymene	t	t	t	t	t	t
<b>1024</b>	<b>limonene</b>	-	t	-	-	-	t
<b>1025</b>	<b>sylvestrene</b>	<b>34.9</b>	<b>12.5</b>	<b>2.0</b>	<b>1.9</b>	<b>1.5</b>	<b>9.5</b>
<b>1025</b>	<b><math>\beta</math>-phellandrene</b>	-	<b>0.7</b>	<b>1.4</b>	<b>1.2</b>	<b>1.0</b>	<b>1.0</b>
1032	(Z)- $\beta$ -ocimene	t	t	t	t	t	t
1044	(E)- $\beta$ -ocimene	t	t	t	t	t	t
1054	$\gamma$ -terpinene	t	t	t	t	t	t
1065	cis-sabinene hydrate	t	t	t	t	t	t
<b>1086</b>	<b>terpinolene</b>	<b>0.6</b>	<b>0.6</b>	<b>0.6</b>	<b>0.5</b>	<b>0.4</b>	<b>0.5</b>
1114	endo-fenchol	t	t	t	t	t	t
1118	cis-p-menth-2-en-1-ol	0.3	0.2	0.3	0.2	0.1	0.2
1122	$\alpha$ -campholenal	t	t	t	t	t	t
1136	trans-p-menth-2-en-ol	0.2	0.2	0.3	0.2	0.1	0.2
1141	camphor	t	0.5	0.5	0.4	0.2	0.3
1145	camphene hydrate	t	t	t	t	t	t
1158	trans-pinocamphone	t	t	t	t	t	t
1160	pinocarvone	t	t	t	t	t	t
<b>1165</b>	<b>borneol</b>	t	<b>1.0</b>	<b>0.8</b>	<b>0.7</b>	<b>0.3</b>	<b>0.6</b>
1174	terpinen-4-ol	t	t	t	t	t	t
1179	p-cymen-8-ol	t	t	t	t	t	t
1186	$\alpha$ -terpineol	0.4	0.4	0.2	t	0.2	0.2
1195	myrtenol	t	t	t	t	t	t
1195	cis-piperitol	t	t	t	t	t	t
1207	trans-piperitol	t	t	t	t	t	0.1
1215	trans-carveol	t	t	t	t	t	t
1218	endo-fenchyl acetate	t	t	t	t	t	t
1226	cis-carveol	t	t	t	t	t	t
1243	piperitone	t	t	t	t	t	t
1254	linalool acetate	t	t	-	t	t	t
<b>1284</b>	<b>bornyl acetate</b>	<b>2.2</b>	<b>5.5</b>	<b>7.0</b>	<b>5.5</b>	<b>1.8</b>	<b>4.5</b>
1342	trans-piperitol acetate	0.5	0.3	0.7	0.4	0.2	0.4
<b>1345</b>	<b><math>\alpha</math>-terpinyl acetate</b>	<b>0.7</b>	<b>0.7</b>	<b>1.1</b>	<b>0.9</b>	<b>0.6</b>	<b>0.8</b>
1374	$\alpha$ -copaene	t	t	t	t	t	t
1387	$\beta$ -bourbonene	t	t	t	t	t	t
1389	$\beta$ -elemene	t	t	t	t	t	t
1407	longifolene	t	t	t	t	t	t
<b>1417</b>	<b>(E)-caryophyllene</b>	<b>2.0</b>	<b>2.4</b>	<b>2.3</b>	<b>2.4</b>	<b>1.8</b>	<b>2.3</b>
1430	$\beta$ -copaene	t	t	t	t	t	t
1439	aromadendrene	t	t	t	t	t	t
1454	$\alpha$ -humulene	0.3	0.4	0.4	0.4	0.3	0.4
<b>1480</b>	<b>germacrene D</b>	<b>9.2</b>	<b>8.9</b>	<b>10.6</b>	<b>9.6</b>	<b>8.7</b>	<b>9.7</b>
1489	$\beta$ -selinene	t	t	t	t	t	t
1500	bicyclogermacrene	0.5	0.7	0.6	0.5	0.7	0.6
1500	$\alpha$ -muurolene	t	t	t	t	0.1	0.1
1508	germacrene A	t	0.2	t	t	0.1	0.1
1513	$\gamma$ -cadinene	t	0.3	t	t	0.3	0.2
1522	$\delta$ -cadinene	0.3	0.6	0.33	0.3	0.5	0.4
1537	$\alpha$ -cadinene	t	t	t	t	t	t
<b>1574</b>	<b>germacrene-D-4-ol</b>	<b>0.3</b>	<b>1.1</b>	<b>0.4</b>	<b>0.3</b>	<b>1.0</b>	<b>0.6</b>
KI	compound	Bulgaria 14738	Bulgaria 14740	Bulgaria 14741	Bulgaria 14737	Bulgaria 14739	Average 15775

1583	caryophyllene oxide	t	0.1	t	0.1	0.1	0.1
1608	humulene epoxide II	t	t	t	t	t	t
1608	$\beta$ -atlantol	t	t	t	t	t	t
1638	epi- $\alpha$ -cadinol	t	t	t	t	t	t
1640	epi- $\alpha$ -muurolol	t	t	t	t	t	t
1645	1-epi-cubenol	t	t	t	t	t	t
1652	$\alpha$ -cadinol	t	0.3	t	0.2	0.3	0.3
1685	germacra-4(15),5,10)14)- trien-1-al	t	t	t	t	t	t
1739	oplopanone	t	0.1	t	t	0.1	0.1
1959	hexadecanoic acid	0.2	0.2	t	t	0.2	0.2
1987	manool oxide	t	t	t	t	t	t
1987	pimara-7,15-diene<iso->	t	t	t	t	t	t
2014	palustradiene	t	t	t	t	t	t
2149	abienol	t	t	0.3	0.2	0.4	0.2
2220	isopimaral	t	t	t	t	0.1	t
<b>2243</b>	<b>palustral (8,13- abietadien-18-al</b>	<b>0.2</b>	<b>0.2</b>	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>
2297	methyl isopimarate	t	t	t	t	t	t
2313	abietal	t	t	t	t	t	t